pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, and xylanase.

REMARKS

Claims 98-123 have been canceled. New claims 124-149 have been added and are pending in the present application. New claims 124-138 are directed to methods for producing a secreted heterologous polypeptide, comprising: (a) cultivating a mutant cell of a parent *Fusarium venenatum* cell under conditions conducive for the production of the secreted heterologous polypeptide, wherein (i) the mutant cell comprises a first nucleic acid sequence encoding the secreted heterologous polypeptide, and (ii) the mutant cell comprises a second nucleic acid sequence which comprises a modification of a cyclohexadepsipeptide synthetase gene, wherein the mutant cell produces less cyclohexadepsipeptide than the parent *Fusarium venenatum* cell when cultured under the same conditions; and (b) isolating the secreted heterologous polypeptide from the cultivation medium. New claims 139-147 are directed to cyclohexadepsipeptide-deficient mutant cells of a parent *Fusarium venenatum* cell, comprising (i) a first nucleic acid sequence encoding a secreted heterologous polypeptide, and (ii) a second nucleic acid sequence comprising a modification of a cyclohexadepsipeptide synthetase gene, wherein the *Fusarium venenatum* mutant cell produces less cyclohexadepsipeptide than the parent *Fusarium venenatum* cell when cultured under the same conditions.

The Advisory Action states that claims 98-123 would be rejected under 35 U.S.C. §112, second paragraph, §112, first paragraph, and §103(a) for the reasons of record. The rejections are respectfully traversed.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. Rejection of Claims under 35 U.S.C. § 112, First Paragraph

The claims stand rejected under 35 U.S.C. § 112, First Paragraph, because (i) the subject matter was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention and (ii) because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make

and use the invention commensurate in scope with these claims. The rejections are respectfully traversed.

The Advisory Action states that Applicant's arguments in regard to the use of conserved regions and the use of Applicant's SEQ ID NO: 1 and 2 to target genes involved in cyclohexadepsipeptide synthesis are not persuasive since the state of art clearly teaches the unpredictability of using sequence homology to isolate polynucleotides/polypeptides of similar function." Applicant respectfully disagrees with this assertion.

In the instant invention, Applicant is simply disrupting or removing a portion of a cyclohexadepsipeptide synthetase gene sequence so expression of the gene is disrupted and no cyclohexadepsipeptide is produced. It is well known in the art that by selecting a conserved or homologous region of a known gene based on sequence comparisons to other similar genes known in the art, a deletion vector can be prepared without knowledge of the corresponding gene sequence in a cell and disrupt that corresponding gene. Moreover, the presence of a cyclohexadepsipeptide synthetase gene in a cell can be determined by employing the known gene, or a portion thereof, as a probe in Southern hybridization analysis under varying stringency conditions. Applicant has shown that the deduced amino acid sequence (SEQ ID NO: 2) of the cyclohexadepsipeptide synthetase gene of SEQ ID NO: 1 shares approximately 59% identity with the cyclohexadepsipeptide synthetase gene (*esyn1*) of *Fusarium scirpi* (Haese *et al.*, 1993, *Mol. Microbiol.* 7: 905-914; DNA sequence listed in EMBL database under accession number Z18755). This sequence comparison indicates there are regions of conserved homology between the sequences at the DNA level, which can be used to construct a disruption or deletion vector for use in another *Fusarium venenatum* cell without any knowledge of the DNA sequence in that cell. This state of art is demonstrated by Herrmann *et al.* (*Molecular Plant-Microbe Interactions* 9: 226-232, 1996) who showed that an internal fragment of the *Fusarium scirpi* cyclohexadepsipeptide synthetase gene was useful in disrupting the *Fusarium avenaceum* cyclohexadepsipeptide synthetase gene without any knowledge of the full nucleic acid sequence of the *Fusarium avenaceum* gene.

Applicant's specification provides a written and enabling description as to how to produce mutant cells from a parent *Fusarium venenatum* cell by deleting or disrupting a nucleic acid sequence encoding a cyclohexadepsipeptide synthetase in the parent cell (see page 5, line 14 to page 8, line 3 and Examples 5 and 6), and how to express a secreted heterologous protein in such a mutant cell (page 11, line 22, to page 17, line 24). From page 5, line 14, to page 7, line 23, Applicant discloses several other procedures well known in the art which can be used to modify the production of cyclohexadepsipeptide in a *Fusarium venenatum* cell. With the

information provided by Applicant in the specification and the knowledge available in the pertinent art, one skilled in the art can construct disruption or deletion vectors for transformation into any *Fusarium venenatum* cell, shown to produce cyclohexadepsipeptide, to disrupt or delete a cyclohexadepsipeptide synthetase gene without knowledge of the gene's sequence. For example, a DNA fragment containing a conserved or homologous region interrupted with a selectable marker or a DNA fragment with a portion of the conserved or homologous region removed by digestion with a restriction enzyme can be used with reasonable predictability to replace the corresponding similar gene via homologous recombination in a *Fusarium venenatum* cell that produces cyclohexadepsipeptide.

Applicant asserts, therefore, that it is well within the skill of the art to make cyclohexadepsipeptide-deficient *Fusarium venenatum* cells using the nucleic acid sequences disclosed in the specification and the prior art without being provided with the corresponding DNA sequences encoding the enzymes involved in the biosynthesis of cyclohexadepsipeptide. The need for isolation of the gene of a *Fusarium venenatum* cell, delineation of the nucleic acid sequence, and a determination of which modifications would lead to deficient production of cyclohexadepsipeptide is not necessary to disrupt or delete a gene involved in the biosynthesis of cyclohexadepsipeptide.

II. Rejection of Claim 114 under 35 U.S.C. § 112, Second Paragraph

Claim 114 stands rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for the reasons of record, *i.e.*, in the recitation of "the mutant cell further comprises one or more modifications of one or more third nucleic acid sequences" because the term "third" relates to a location or position within a structure and it is unclear how two or more sequences can occupy the same position or location. Claim 114 has been cancelled, but new claims 136 and 148 recite essentially the same language. This rejection is respectfully traversed.

Applicant previously pointed out that the term "third nucleic acid sequence" is defined by Applicant on page 8, line 22, to page 9, line 4, of the specification. The "third nucleic acid sequence" does not relate to a location or position within a structure, but relates simply to a third nucleic acid sequence encoding a protein that may be detrimental to the production, recovery, and/or application of the heterologous polypeptide of interest. Proteases are an example well known in the art. Moreover, the claim recites "the mutant cell <u>further</u> comprises one or more third nucleic acid sequences." The term "further" means in addition to the first and second nucleic acid sequences, not in place of first and second nucleic acid sequences. Applicant asserts, therefore, that the term "third" is not vague and indefinite.

For the foregoing reason, Applicant submits that the rejection under 35 U.S.C. § 112, second paragraph, has been overcome and respectfully request reconsideration and withdrawal of the rejection.

III. Rejection of Claims under 35 U.S.C. § 103(a)

Claims 98-103 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over by Herrmann *et al.* (*Molecular Plant-Microbe Interactions* 9: 226-232, 1996) in view of Tsuchiya *et al.* (*Appl. Microbial. Biotechnol.* 40: 327-332, 1993) for the reasons of record. This rejection is respectfully traversed.

The claimed methods of producing a secreted heterologous polypeptide in a cyclohexadepsipeptide-deficient *Fusarium venenatum* cell would not be obvious to one of ordinary skill in the art, based upon the teachings cited by the Advisory Action. None of the references, either alone or in combination, suggest or teach one of ordinary skill in the art methods of producing a secreted heterologous polypeptide in a cyclohexadepsipeptide-deficient *Fusarium venenatum* cell. Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination.

The Advisory Action recognizes that "obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art."

Applicant's arguments of record indicate that Herrmann *et al.* and Tsuchiya *et al.*, alone or in combination, provide no teaching or suggestion of producing a secreted heterologous polypeptide in a cyclohexadepsipeptide-deficient *Fusarium venenatum* cell, as claimed herein. Since Herrmann *et al.* and Tsuchiya *et al.*, alone or in combination, provide no such teaching or suggestion, there is no motivation to use the mutant filamentous fungal cell of Herrmann *et al.* to produce secreted heterologous proteins. The knowledge generally available to one of ordinary skill in the art for producing a secreted heterologous polypeptide in a cyclohexadepsipeptide-deficient *Fusarium venenatum* cell did not exist until Applicant's disclosure.

The Office Action of April 23, 2002, stated that "[o]ne of ordinary skill in the art has a reasonable expectation of success at being able to produce heterologous secreted proteins such as proteases, in the host of Herrmann *et al.* because transformation of filamentous fungal cells with a plasmid containing the DNA encoding the heterologous protein under the control of a promoter which would allow secretion is well known in the art and Tsuchiya *et al.* teach a method

to secrete a heterologous protease in a filamentous fungal cell." Applicant disagrees with this contention because neither Herrmann *et al.* nor Tsuchiya *et al.* teach or suggest that a cyclohexadepsipeptide-deficient *Fusarium venenatum* can be used as a host cell to produce a secreted heterologous polypeptide. Herrmann *et al.* teach a method for reducing the virulence of *Fusarium avenaceum*, but makes no mention of using such a strain or a cyclohexadepsipeptide-deficient *Fusarium venenatum* for producing a secreted heterologous polypeptide. Tsuchiya *et al.* teach the use of an *Aspergillus oryzae* strain for expressing a foreign protein, but makes no mention of cyclohexadepsipeptide-deficient *Fusarium venenatum* strains for such a purpose. Even in combination, there is no suggestion that a cyclohexadepsipeptide-deficient *Fusarium venenatum* can be used as a host cell to produce a secreted heterologous polypeptide.

The cited references do not contain the requisite teaching, and therefore cannot be combined to support the obviousness rejections of the present claims. For the foregoing reason, Applicant submits that the rejections under 35 U.S.C. § 103(a) have been overcome and respectfully request reconsideration and withdrawal of the rejections.

IV. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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